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Effects of generations in captivity and elevated rearing temperature on Ontario hatchery brook trout (*Salvelinus fontinalis*) fry quality and survival

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Abstract With rising environmental temperatures causing concern for the status of freshwater fishes, captive breeding programs may become increasingly important for conservation efforts as well as to support fisheries. Although captive broodstocks provide reliable gamete sources for production stocking, prolonged generations under hatchery conditions can result in changes to fishes as they acclimate to captive settings (domestication) — for example, reduced plasticity due to homogenous captive environments. We assessed the effects of rearing temperature and number of generations spent in captivity on the survival and quality (indicated by lack of malformations) of long-term ($F_{>25}$) and newly captive (F_1) strains of Ontario hatchery brook trout (*Salvelinus fontinalis*) with shared genetic history. Elevated temperatures decreased likelihood of survival between the pre-exogenous feed and emergent fry stages and had a greater impact on fry quality (rate of malformations) on F_1 fish compared with $F_{>25}$ fish, suggesting no reduction in plasticity due to prolonged captivity. However, overall survival between F_1 and $F_{>25}$ fish was not different. The combined effects of elevated rearing temperatures and number of hatchery generations suggest that (selection) changes due to captivity can occur rapidly even under benign conditions and that additive stressor effects of captivity and temperature have the potential to impact newly established strains.

Keywords Climate change · Salmonid · Offspring survival · Hatchery rearing · Domestication · Rearing temperature

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Introduction

Climate change is increasing air and water temperatures globally (Creed et al. 2017), and freshwater aquatic ecosystems are particularly vulnerable due to their dependency on air and precipitation temperatures combined with higher levels of human use (Woodward et al. 2010; Stitt et al. 2014; Bunn 2016; Cook et al. 2018a, b; Hanna et al. 2018; Potts et al. 2021). Effects are especially impactful on cold-water adapted freshwater species, such as

salmonids. Due to a combination of elevated water temperatures and loss of suitable habitat, salmonids have shown declines in abundance that are 64% higher than projected (Lake et al. 2000; Creed et al. 2017; Myers et al. 2017; Desforges et al. 2022). Specifically, elevated temperatures have been shown to impact the reproductive performance and survival of salmonids via multiple mechanisms: increased metabolic rates forcing energetic reallocation to homeostasis versus reproduction (Fenkes et al. 2016), the inhibition of production of hormones required for gonadogenesis reducing the overall reproductive potential of fishes (King et al. 2007; Pankhurst and King 2010), and accelerated and subsequent underdeveloped hatch state of eggs (Hayes et al. 1953; Baird et al. 2002; McDermid et al. 2012). As embryonic and reproductive stages are the most thermally sensitive (Dahlke et al. 2020), increased temperatures pose a threat to reproduction and recruitment in cold-adapted salmonid populations.

Captive breeding and supplemental stocking of freshwater fishes have often been employed as a common restoration tool to reverse or mitigate population declines (George et al. 2009; Houde and Pitcher 2016; Cote et al. 2021), particularly in freshwater fishes (Ward et al. 2008) such as salmonids (Solar 2009). Captive breeding programs require the cultivation of breeding adults through assigned mating, highly controlled environments, and strict nutrition to produce offspring which are eventually used for stocking events (Bromage et al. 1992; Carrillo et al. 2000; Morissette et al. 2018; Olk et al. 2019; Kuciński and Fopp-Bayat 2021). Progeny quality is quantified using metrics such as egg fertilization, hatching success, survival through to the emergent fry stage (as defined by the period before the first exogenous feed), and fry quality (as determined by the absence of malformations; Bromage et al. 1992; Carrillo et al. 2000). Nevertheless, broodstocks can inadvertently undergo multiple generations of selection to perform optimally in captive settings as a result of genetic, physiological, and behavioural adaptations to hatchery conditions (Liu et al. 2015). However, because of the higher survival rate of captive individuals (85–95% vs 1–5% of wild), greater instances and detections of malformations can also result, as many developmental anomalies commonly seen in hatcheries are thought to have a genetic basis and malformed fish are surviving further into the emergent fry stage (Ihssen 1978; Branson and Turnbull 2008).

Additionally, long-term adaptation to captivity can result in captive individuals losing plasticity as a result of relaxed selection pressures in the hatchery (Mason et al. 2013; Milla et al. 2021); for example, in their responses to "irrelevant" stressors (i.e., antipredator behaviour in *Salmo salar*; de Mestral and Herbinger 2013), temperature stress (i.e., metabolic rate in *Salmo trutta*; Watz 2019), and life-history traits (i.e., age at maturity in *Hypomesus transpacificus*; LaCava et al. 2023). In essence, hatchery practices relax wild selection pressures and thereby remove the negative fitness consequences; as a result, maintaining plastic responses to now-stable environmental stimuli is costly with associated tradeoffs (LaCava et al. 2023). This loss can prove deleterious for individuals slated for release.

To mitigate or offset losses in genetic and/or phenotypic plasticity of hatchery strains, captive populations are periodically refreshed by genetic infusions from related wild populations by either crossbreeding or creating new genetic lines (Frankham 2008; Fraser 2008). This serves to provide relief from domestication selection, reduce inbreeding potential, minimize effects of kinship, and help to prevent outbreeding depression upon stocking (Fisch et al. 2015). However, there is evidence that cold-freshwater fishes can experience rapid selection in a captive environment with these changes showing up in as little as one generation (Christie et al. 2012, 2016; Fraser et al. 2019). In addition to increasing genetic diversity (Rodewald 2013), hatchery programs can also manipulate or enrich hatchery environments for broodstock and production fish to maximize post-stocking success. Especially important is addressing impacts of climate change on cold-water fish species, and hatcheries provide opportunities for experimental evaluation of elevated rearing temperatures on offspring survival (Molony et al. 2004; Pankhurst and King 2010; McDermid et al. 2012) and quality (Yamamoto et al. 1996; Fraser et al. 2015; Fjelldal et al. 2016). For instance, elevated rearing and incubation temperatures are used to determine whether fish can be "phenotypically programmed" to deal with increased stressors upon release (e.g., climate change stressors; Roberts et al. 2014; Cook et al. 2018b), and elevated temperatures have been used to deliberately induce certain malformations for study (e.g., Atlantic salmon

(*Salmo salar*) × Arctic charr (*Salvelinus alpinus*) hybrids; Fjelldal et al. 2016). From a practical standpoint, elevated temperatures are also used by hatchery managers to increase egg survival and fry growth rates and therefore decrease rearing times (Marten 1992; Baird et al. 2002), as well as manipulate hatch times (Weber et al. 2016) to increase production efficiency. Despite possible benefits, this thermal stress may cause a reduction in survival and increase in temperature-related malformations (e.g., blue sac disease in several charr species (Ihssen 1978), spinal deformities in Atlantic salmon (Fraser et al. 2015), and possibly negate the advantages of priming and/or accelerating growth (e.g., carry-over effects at later-life history stages post stocking; Harbicht et al. 2021).

Most studies on egg survival and quality have focused on comparing responses of wild-type individuals with either short-term or long-term captive strains (Milla et al. 2021). By contrast, relatively few studies have compared newly established and long-term captive individuals; those that do tend to focus on post-release phenotypes rather than survival within a hatchery setting (Kostow 2004; although see Howell et al. 2022), or manipulation of spawning adults (Cieśla et al. 2014). Since early development is a vulnerable life history stage for fishes, particularly with respect to temperature stressors (Dahlke et al. 2020), characterizing the integrated effects of number of hatchery generations and performance under stress during early life can aid in predicting reproductive output and contributions to populations upon stocking (Haponski and Stepien 2016). Here, we used a two-by-two experimental design to investigate the effects of elevated rearing temperatures and number of generations in captivity on newly-captive (F_1) and long-established (domesticated, $F_{>25}$) hatchery strains of brook trout (*Salvelinus fontinalis*) with shared ancestry (Fig. 1). Specifically, we compared how domesticated and newly captive strains fared under normal hatchery conditions versus a stressful environment, assessing egg survival and fry quality traits in both strains in benign and temperature-stressor conditions. We predicted that elevated rearing temperatures would significantly reduce the likelihood of survival in both captive strains, with domesticated fish being more thermally sensitive due to numerous generations of rearing at constant hatchery temperatures. We also expected that while temperature would increase malformation rates, domestic fish would show higher instances of malformations and lower quality than the F_1 strain under both temperature treatments due to unintentional inbreeding and suboptimal phenotype accumulation that is known to occur in hatchery settings (Crozier et al. 2011). Should selection to hatchery conditions begin to act within one generation, we expected to see no variation in survival between long-term captive and newly captive fish populations under normal captive conditions.

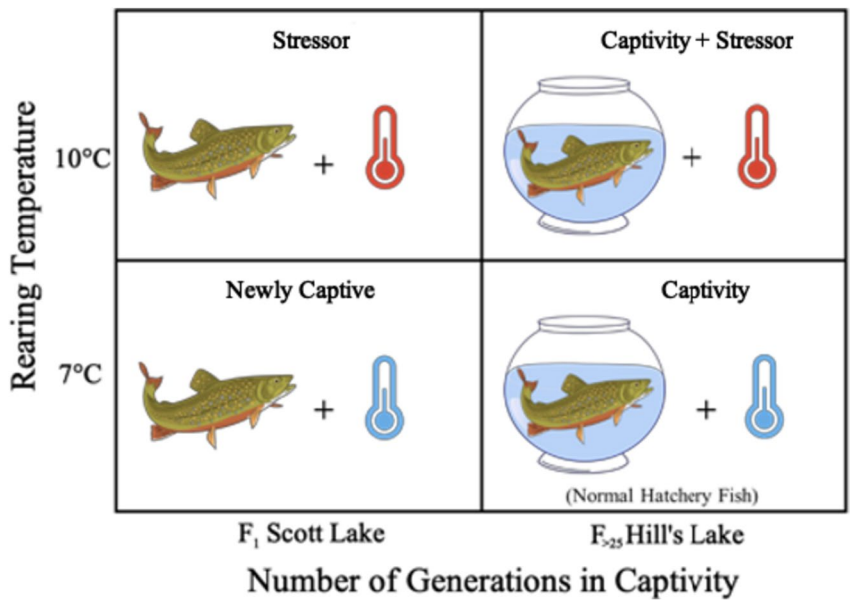

Fig. 1 Experimental design to assess potential impacts of captivity. Eggs from each family and population were raised at two temperatures

Methods

Fish origins

We used fish from two brook trout strains held at the Ontario Ministry of Natural Resources and Forestry (OMNRF) Codrington Fisheries Research Facility. The Hill's Lake strain is a provincial production broodstock that has been in the Ontario hatchery system for more than 25 generations with some mid-twentieth century genetic input from wild sources and is considered to be heavily domesticated (OMNRF 2005). The Scott Lake strain is a newly captive wild-origin research strain sourced from Scott Lake (45.4856°N, 78.7237°W) in Algonquin Park, Ontario. The wild brook trout population in Scott Lake was supplementally stocked with the Hill's Lake strain multiple times from 1954 to 1983 (more than 14,000 yearlings over 15 stocking events: OMNRF unpubl. data) and shows extensive genetic introgression from the historical stocking (Harbicht et al. 2014; OMNRF unpubl. data). Recent analyses suggest that the population is functionally a naturalized hatchery population with no remaining native ancestry evident, despite ten generations of wild recruitment since the last stocking event (OMNRF, unpubl. data). Wild-origin gametes from Scott Lake were brought into the Codrington facility and reared in captivity for one full generation before the start of our experiment (i.e., parents used for making family crosses in this study originated from a wild spawn collection from Scott Lake and were brought into the hatchery system as fertilized eggs). Thus, the domesticated Hills Lake strain and wild-origin Scott Lake strain are very similar genetically and differ primarily in their number of generations spent in a hatchery environment and number of generations removed from the wild. Both strains are kept under common rearing conditions at the OMNRF Codrington Fisheries Research Facility (Codrington Ontario, 44.15°N, 77.80°W).

Breeding and rearing design

Fertilized eggs were obtained from spawning adult fish at the Codrington Fisheries Research Facility. Between 15 and 29 November 2019, ten male and ten female 4-year old adult brook trout were used from the Hill's Lake broodstock. The same number of male and female adult trout of the same age were used from F_0 generation Scott Lake brook trout (brought in as wild-collected egg families). Adults were reared at ambient climatic conditions using a flow-through surface water system with an average ambient water temperature of 7.17 °C (see Howell et al. 2022). Five two-by-two fertilization crosses were performed within each population (i.e., five replicates of two females each crossed with two males). No siblings were used in the same 2×2, and no identical crosses were used. To collect gametes, adults were anesthetized using MS222 (Tricaine®-S Topical Anesthetics). Each adult had their pre-spawn mass recorded and photo taken. Eggs were dry-spawned into two 500 mL glass jars by applying slight abdominal pressure to the female and fertilized at the hatchery. For the 2×2 cross, milt from two males was expressed via slight abdominal pressure and collected using disposable pipettes and partitioned across the two females' jars (i.e., there was no pooling across males or females). Hatchery water was used to activate eggs and milt; fertilization was stimulated by gently swirling the jar of eggs. Egg families were then left to begin water hardening for approximately 10 min, then disinfected with a 50-mg/L solution Ovadine® according to OMNRF protocol (Community Hatcheries Program 2019), allowed to water harden for half an hour, rinsed with hatchery water three times, and transported to the University of Windsor's Great Lakes Institute for Environmental Research aquatics facility in coolers on ice. Upon arrival eggs were disinfected a second time using dechlorinated Culligan® filtered water, following OMNRF procedure, before being placed into the incubation stacks. Families were split by equal volume into four egg cups (two replicates per temperature treatment (mean: 463 ml ± 79.456, Range: 341–627 ml; See Appendix A for sample sizes), that were randomized for position in the incubation stacks. The number of eggs transferred per family was not consistent across all females and families as the female's entire volume of eggs was used, and egg size was variable. Eggs were fertilized in three spawning batches across 3 weeks based on availability of spawning adults.

Within each strain, offspring were raised on two distinct temperature regimes: 7 °C — near the optimal temperature for brook trout eggs (Hokanson et al. 1973) and similar to temperatures at the Codrington hatchery (Howell et al. 2022), but within the physical capabilities of our rearing system, and 10 °C — as

this difference has been found previously to produce significant survival differences in brook trout without approaching the TL_{50} of 12.7 °C (Hokanson et al. 1973). An increase of 3 °C also represents future water temperatures in accordance with current climate change projections (Austin and Colman 2007; Trumpickas et al. 2009; van Vliet et al. 2013). We chose to rear fish on constant temperature regimes as this is a common practice in hatcheries and reflective of conditions at the Codrington hatchery (Howell et al. 2022), and cyclic water temperatures have been linked to increase stress and reduced survival in fish embryos (Meeuwig et al. 2004), thereby adding confounding variables to our experimental design. Each temperature treatment was arranged with its own recirculated, UV-sterilized, and dechlorinated water system. During incubation, water changes of approximately 15 gallons were completed each day. Water quality tests for ammonia, nitrates, and nitrites were completed weekly, while pH, dissolved oxygen, and temperature were monitored daily using HOBO® temperature loggers (ONSET®) and a LabQuest®2 unit (Vernier Connections™). The daily average temperature from the HOBO® logger was calculated to determine accumulated thermal units (ATU) which allows for the standardization between temperature systems, as development and growth in fish is highly temperature dependent (Chezik et al. 2014).

Survival tracking

Dead eggs or embryos were cleared from the incubation cells every other day and number of removed eggs was recorded. Mortalities were sorted into three categories: eyed, pre-exogenous feed, and emergent fry. Stages were chosen based upon relevance to hatchery rearing methods (where eggs are removed if they fail to eye or hatch) and to account for sensitive developmental stages (Hoitsy et al. 2012). Any unfertilized eggs or those that died immediately post fertilization (as indicated by failure to eye) were considered to be not-eyed. All unfertilized eggs were removed three days post fertilization in the first removal. "Eyed" mortalities were embryos that died either before hatch or eyed eggs that never survived hatching. To be considered dead at the pre-exogenous feed stage, an embryo had to be at least 70% (estimated visually) out of their egg with the shell not restricting their movement. After a minimum of 326 ATU (when the majority of all eggs were eyed based on visual inspection and standardized between temperature treatments), any remaining non-eyed eggs were considered dead and removed. Survival percentage was calculated using the total number of fertilized eggs per replicate (mean 403 ± 97, range 175–688 eggs). To control for density effects on hatching rates, the number of eggs per sample was counted from photos of incubation cups using the ImageJ multipoint tool (Rasband 1997–2018).

Visible malformations were tracked as a secondary measure of survival as malformed individuals are culled from stocking populations (Ihssen 1978; Branson and Turnbull 2008; Fraser et al. 2015). Malformations were categorized as spinal (curved or curled spine), gametal fusion (dicephalous, tricephalous, anadidymus type, katadidymus type, anakatadidymus type, and parasitic), or blue sac disease (Ihssen 1978; Laale 1984; Toften and Jobling 1996; Yamamoto et al. 1996); with each category representing malformations with both genetic and environmental origins. We also tracked individuals who had more than one malformation type, labelled as multiple malformations (Fig. 2).

Statistical methods

We completed all statistical analyses using R version 4.1.0 (R Core Team 2021). Using the *fullfact* package (Houde and Pitcher 2016), we performed a binary expansion on survival data at each developmental stage, beginning at fertilized (with non-fertilized eggs removed from analysis), giving individuals alive in a given stage a score of 1 and dead individuals a score of 0. We analyzed this binary survival data with a generalized linear mixed model using a binomial distribution with a log link function, with temperature (categorical: 7 °C and 10 °C), strain (categorical: $F_{>25}$ Hill's Lake (domestic) and F_1 Scott Lake (F_1 captive)), stage (categorical, 3 levels: eyed, pre-exogenous feed, emergent fry), strain by temperature interaction, temperature by stage interaction, and strain by stage interaction as main effects. We also included estimated initial egg number per family as a covariate in the model (continuous, range 341–627) to control for density effects. Family identification number (categorical, 1–40 families), female (dam) identity (categorical, 1–20 dams), male (sire) identity (categorical, 1–20 sires), dam ID × sire ID (family) interaction,

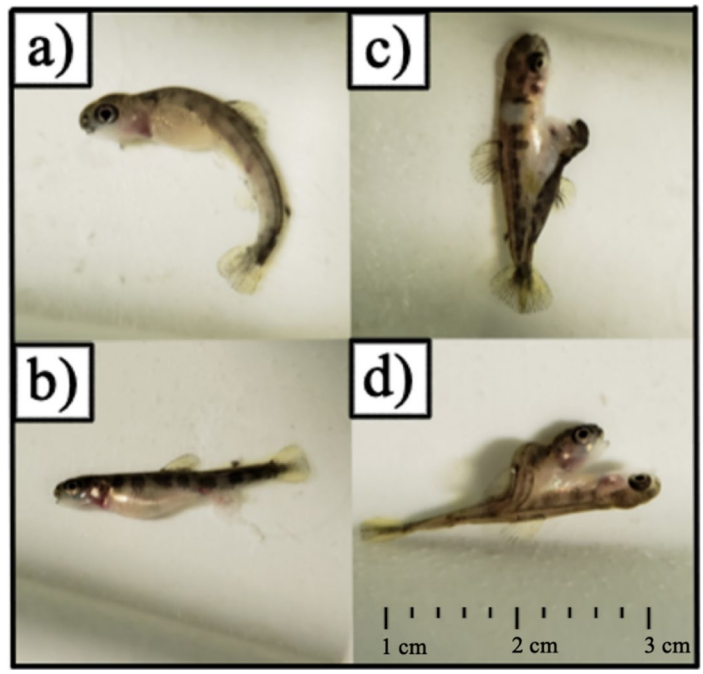

Fig. 2 Pictures of malformed individuals in each category **a** spinal malformations, **b** blue sac disease, **c** gametal fusion, **d** multiple malformations (e.g., spinal malformation and gametal fusion pictured here). All individuals are shown in the emergent fry stage

fertilization block ID (categorical, 1–10 fertilization blocks), and incubation cell identity (i.e., replicate; categorical, 1–12 cells) nested in incubation tray (categorical, 1–14 trays) were included as random effects.

For assessing the likelihood of hatched individuals with developmental malformations, we performed the same binary expansion giving "no-malformation" fish a score of 1 and "malformation" fish a score of 0. We analyzed malformation data with a generalized linear mixed model using a binomial distribution with a log link function with temperature, strain, and their interaction as fixed effects, including egg number as a covariate and using the same random-effects structure as above. To analyse the occurrence of specific types of malformations among fish expressing malformations, we used a negative binomial model as the count data were overdispersed. In addition to the fixed- and random effects structure used above, malformation type (categorical: spinal malformations, bluesac disease, gametal fusion, presence of multiple malformations) was included, along with the following interactions: strain by temperature interaction, strain by malformation type interaction, and temperature by malformation-type interaction.

Following the methods defined in Capelle et al. (2017) and Warriner et al. (2020), we analysed model fit using the restricted maximum likelihood approach (Zuur et al. 2009). When a significant interaction was present ($p \leq 0.05$), all interactions were retained and pairwise comparisons between significantly interacting variables were made using Tukey's HSD. Both significant and insignificant interactions were verified by likelihood ratio test (LRT) model comparison using ANOVA. Non-parental random effects were individually removed from the models if they were non-significant, as assessed by the same method. Dam, sire, and family effects were retained regardless of their significance to account for their contributions to overall model variance. We assessed the normality of assumption of model deviance residuals of our binomial regressions by visual inspection of quantile–quantile residual plots and histogram of residuals. Our negative binomial model residuals were additionally assessed using the Kolmogorov–Smirnov test (Hartig and Lohse 2022). Packages used in performing statistical analyses include *fullfact* (Houde and Pitcher 2016) to convert continuous into binary data, *MASS* (Venable and Ripley 2002) for linear mixed models and nonlinear least squares, *glmmTMB* (Brooks et al. 2023) to perform negative binomial regression, *lme4* (Bates et al. 2023) and *lmerTest* (Kuznetsova et al. 2020) to run GLMMs, *emmeans* (Lenth et al. 2023) for post hoc comparisons, *DHARMa* (Hartig and Lohse 2022) to

test for overdispersion and assess non-Pearson residuals, *ggeffects* (Lüdecke et al. 2023) for model predicted plots, and *ggplot2* (Wickham et al. 2023) for visualizations.

Results

Survival analysis

Temperature significantly interacted with developmental stage to affect survival of brook trout eggs ($p \leq 0.001$ $\chi^2 = 174.5$) (Table 1). Under elevated temperatures, pre-exogenous feed and emergent fry stages were more sensitive, exhibiting a decrease in survival in comparison to these stages reared under current conditions (Fig. 3). Temperature and strain had no significant interaction effect on survival ($p = 0.493$; $\chi^2 = 0.113$). Temperature and stage individually had significant effects on survival (temperature $p = 0.028$, stage $p \leq 0.01$), while strain alone had no effect on embryo survival to exogenous feed ($p = 0.902$). Egg density did not significantly impact the likelihood of survival ($p = 0.796$, $\chi^2 = 0.062$). Random effects of dam ($\chi^2 = 21.448$; $p \leq 0.001$), family ($\chi^2 = 12.306$; $p \leq 0.001$), and incubation cell nested within tray ($\chi^2 = 731.93$; $p \leq 0.001$) significantly contributed to variance in survival. Sire was not a significant random effect ($\chi^2 = 0.485$; $p = 0.481$). For model results summarized, see Appendix A.1.

Table 1 Percent survival including standard error between treatments and across developmental stages. Sample size (*N*) is reflective of total fertilized eggs for given a treatment group. For sample sizes at each stage see Appendix B

Strain	Temperature treatment	Eyed	Pre-exogenous Feed	Emergent fry	*N*
$F_{>25}$ *Hill's*	7 °C	0.887 ± 0.029	0.992 ± 0.002	0.995 ± 0.004	18,239
	10 °C	0.865 ± 0.034	0.980 ± 0.006	0.989 ± 0.003	18,130
F_1 *Scott*	7 °C	0.892 ± 0.030	0.985 ± 0.006	0.988 ± 0.004	14,934
	10 °C	0.866 ± 0.036	0.961 ± 0.002	0.982 ± 0.006	14,218

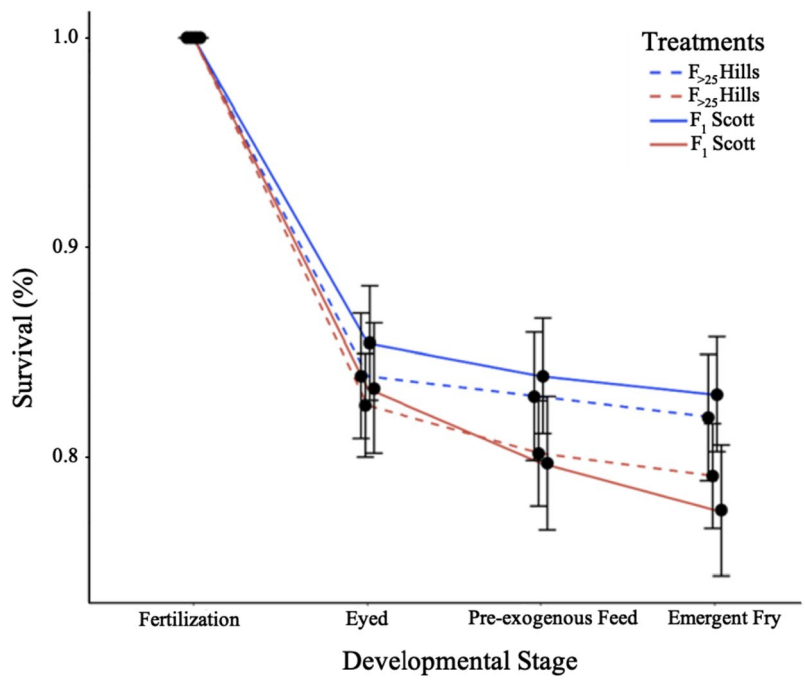

Fig. 3 Percent survival (means ± standard deviation) across all developmental stages for both strains and temperature treatments. Elevated temperatures significantly lowered survival between the pre-exogenous feed- and emergent fry stages in both strains. The eyed stage was unaffected. See Appendix B for sample sizes

Instances of emergent fry with developmental malformation

Temperature and strain interacted to significantly affect the likelihood of malformations in brook trout ($\chi^2 = 5.589$, $p = 0.013$). Under current and elevated temperatures, $F_{>25}$ Hill's Lake and F_1 Scott Lake did not experience different malformation likelihoods (7 °C: Tukey's HSD $p = 0.690$; 10 °C: Tukey's HSD: $p = 0.913$). While $F_{>25}$ Hill's Lake fish did not experience significant change in malformation rates *between* temperature treatments (Tukey's HSD: $p = 0.539$), F_1 Scott Lake fish did experience a significant increase in their likelihood of malformations from current to elevated temperatures (Tukey's HSD: $p \leq 0.001$). Temperature ($p = 0.181$) and strain ($p = 0.272$) were not significant as fixed effects (Fig. 4). Female egg density had no significant impact on the likelihood of malformations ($\chi^2 = 0.626$; $p = 0.419$). Random effects of dam ($\chi^2 = 9.914$; $p = 0.007$) and incubation cell nested in tray ($\chi^2 = 149.6$; $p \leq 0.001$) significantly contributed to variance. Family ($\chi^2 = 0.756$; $p = 0.685$) and sire ($\chi^2 = 1.197$; $p = 0.550$) did not significantly contribute to variation. See Appendix A.2 for model summary.

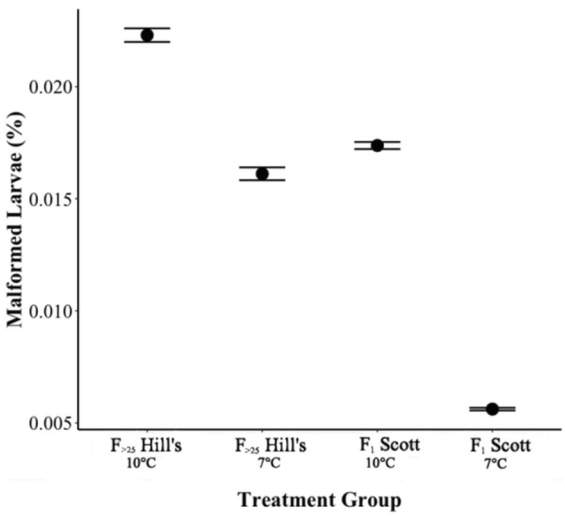

Fig. 4 Percent (means ± standard deviation) of malformed individuals (regardless of type) between treatment groups. F_1 Scott Lake fish were significantly more likely to exhibit malformations due to increased temperatures. See Appendix B for sample sizes

Malformations — number and type

Temperature and strain significantly interacted to influence the number of malformations exhibited ($\chi^2 = 8.062$; $p \leq 0.001$). Type of malformation significantly interacted both with temperature ($\chi^2 = 7.846$; $p = 0.020$) as well as strain ($\chi^2 = 20.61$; $p = 0.004$) to influence number of malformations expressed. Temperature regime ($p = 0.143$) and strain ($p = 0.802$) as main effects did not significantly impact the type of malformations exhibited (Fig. 5). Egg density had no effect on number of malformations ($\chi^2 = 0.066$; $p = 0.797$). Replicates (i.e., incubation cell ID nested in tray ID) significantly contributed to model variance ($\chi^2 = 4.366$; $p = 0.037$). Random effects of dam ($\chi^2 = 2.379$; $p = 0.123$), sire ($\chi^2 = 0$; $p = 0.999$), and family ($\chi^2 = 2.275$; $p = 0.132$) did not contribute significantly to variance. With respect to the total number of malformations expressed, under both temperature regimes $F_{>25}$ Hill's Lake and F_1 Scott Lake fish showed no significant differences between number of fish with malformations (Tukey's HSD: F_1 Scott Lake- $F_{>25}$ Hill's Lake 7 °C $p = 0.291$; F_1 Scott Lake- $F_{>25}$ Hill's Lake 10 °C $p = 0.921$). However, F_1 Scott Lake fish had greater numbers of malformed individuals on elevated compared to current temperatures (Tukey's HSD: $p \leq 0.001$), while $F_{>25}$ Hill's Lake fish showed no significant changes to the number of malformed individuals between temperatures (Tukey's HSD: $p = 0.838$). For ease of interpretation of the interaction effects of mutation type, we report further findings within each temperature treatment. See Appendix A.3 for model results summarised.

Current temperature

When reared on current temperatures, $F_{>25}$ Hill's Lake fish showed no significant differences between specific types of malformations (Tukey's HSD: blue sac—fusion $p = 1.00$, blue sac-spinal $p = 0.287$, fusion-spinal $p = 0.843$); however, significantly fewer individuals expressed multiple malformations (Tukey's HSD: blue sac-multiple $p = 0.004$, fusion-multiple $p = 0.002$, multiple-spinal $p \leq 0.001$). F_1 Scott Lake fish had similar numbers of spinal malformations and blue sac disease (Tukey's HSD: $p = 1.0$), both of which occurred more than gametal fusion (Tukey's HSD: spinal-fusion, $p = 0.013$ blue sac-fusion $p = 0.013$) and multiple malformations

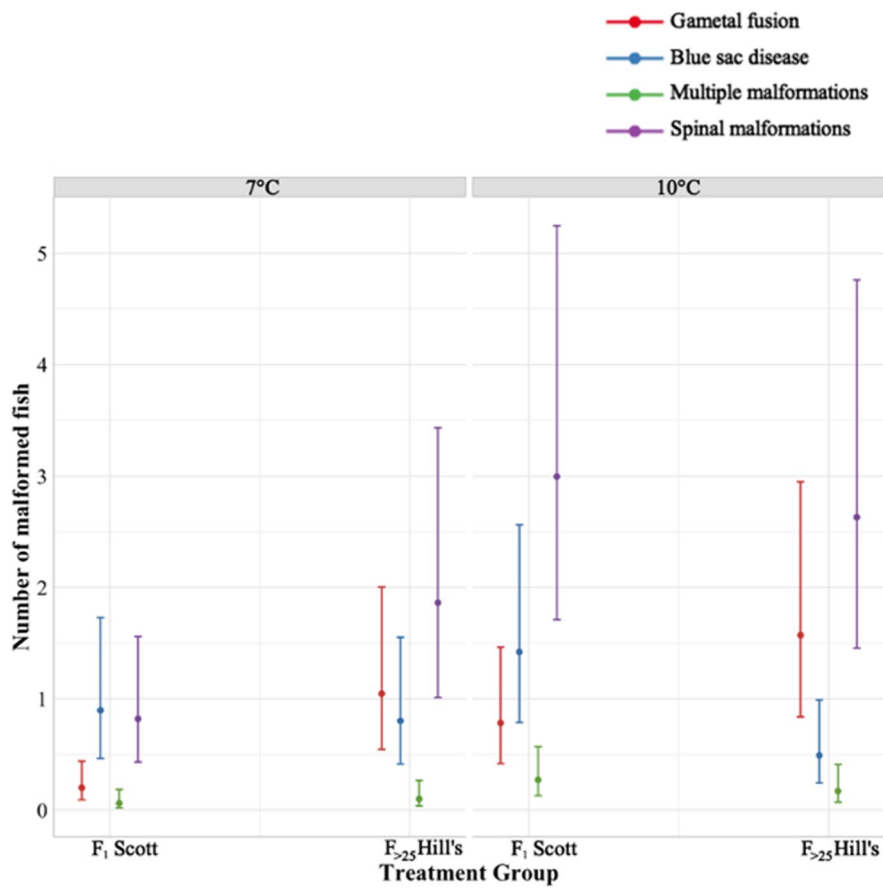

Fig. 5 Model predicted values ± 95% confidence interval of the number of malformed individuals by type for two different strains — $F_{>25}$ Hill's Lake and F_1 Scott Lake reared on two temperature regimes: elevated (10 °C) and current (7 °C). Elevated temperatures increased instances of malformation and the $F_{>25}$ Hill's Lake strain showed greater differential expression of the types of malformations. F_1 Scott Lake fish experienced a greater increase in the number of malformations under elevated versus current rearing temperature

(Tukey's HSD: spinal-multiple $p \leq 0.050$, blue sac-multiple $p \leq 0.001$); the number of fish exhibiting gametal fusion and multiple malformations did not differ (Tukey's HSD: $p = 2.076$).

Elevated temperature

$F_{>25}$ Hill's Lake fish reared on elevated temperatures now showed significant differences between malformation types with significantly more individuals having spinal and fusion malformations than blue sac disease (Tukey's HSD: blue sac-fusion $p = 0.043$, blue sac-spinal $p \leq 0.001$), and significantly fewer individuals had multiple malformations than fusion and spinal malformations (Tukey's HSD: fusion-multiple $p \leq 0.001$, spinal-multiple $p \leq 0.001$). There was no significant difference between individuals with blue sac disease and those with multiple malformations (Tukey's HSD: $p = 0.593$). Similarly, there was no difference between the number of individuals exhibiting spinal malformations and those showing gametal fusion (Tukey's HSD: $p = 0.985$). For fish from the F_1 Scott Lake strain raised on elevated temperatures, spinal malformations were more common than gametal fusion and multiple malformations (Tukey's HSD: spinal-fusion $p \leq 0.001$, spinal-multiple $p \leq 0.001$) but again no different than blue sac disease (Tukey's HSD: spinal-blue sac $p = 0.260$). Blue sac disease was significantly more common than both gametal fusion and multiple malformations (Tukey's HSD: blue sac-multiple $p \leq 0.001$, blue sac-fusion $p = 0.832$), while gametal fusion was not significantly more common than any other malformation type (Tukey's HSD: fusion-multiple $p = 0.240$) (Table 2).

Discussion

Overexploitation, habitat loss and fragmentation, and climate change are causing increased rates of declines in cold-water adapted species such as salmonids (Myers et al. 2017). To combat the trends in

Table 2 Number of malformations by category (including standard error) between treatments and across strains. Sample size (*N*) is reflective of total malformed larvae for a given treatment group. For sample sizes at each stage see Appendix A

		Spinal malformations	Blue sac disease	Gametal fusion	Multiple malformations	*N*
Strain	$F_{>25}$ *Hill's*	2.203 ± 0.592	0.624 ± 0.188	1.276 ± 0.363	0.129 ± 0.051	456
	F_1 *Scott*	1.56 ± 0.423	1.122 ± 0.312	0.395 ± 0.125	0.128 ± 0.051	313
Temperature treatment	*7 °C*	1.230 ± 0.291	0.843 ± 0.212	0.457 ± 0.128	0.077 ± 0.035	260
	10 °C	2.794 ± 0.593	0.831 ± 0.203	1.103 ± 0.258	0.214 ± 0.07	509

decreasing populations, captive breeding programs have been put into place (Rytwinski et al. 2021; Desforges et al. 2022). Despite the reduced productivity of released hatchery fish to wild populations (Christie et al. 2014; O'Sullivan et al. 2020), disproportionate population reductions in salmonids due to climate change and other anthropogenic factors (Myers et al. 2017) create a continued need for captive broodstocks to produce adequate numbers of individuals (Kerr 2000). These programs can nevertheless introduce a new suite of initial selection pressures upon introduction to captivity, or long-term selection issues resulting in domestication (Farquharson et al. 2021), with changes occurring at rapid paces, and in some instances, as little as one captive generation (Fraser 2008; Fisch et al. 2015; Christie et al. 2016; Fraser et al. 2019). Temperature stressors (intentional hatchery practices for conservation and/or production efficiency) along with captive-selection pressures are known to interact and further reduce fitness of salmonids when released into wild settings (Kostow 2004). In our study, we sought to investigate the interactive effects of rearing temperature and the number of generations in captivity on the survival and instances (and diversity) of malformations in emergent fry brook trout originating from a provincial hatchery station bred for stocking. The lack of significant differences in egg survival between strains suggest that this trait may not have been significantly altered by multiple generations in captivity, even in the presence of a stressor. In addition, the newly captive strain exhibited reduced quality of emergent fry fish under elevated rearing temperatures, potentially reflecting reduced plasticity in the Hill's Lake captive population after many generations in captivity. With these findings, we posit that in our study, temperature is a greater driver of any changes to brook trout fry and the benefits of fewer generations in captivity are marginally reduced (Fraser et al. 2019) at early life stages; however, longer-term changes may be more evident and require further research.

Survival under elevated rearing temperatures

In accordance with our original predictions, the elevated rearing temperature significantly reduced the likelihood of survival, particularly between the pre-exogenous feed and emergent fry stages. Survival decreased from 83.5% at 7 °C incubation to 79.7% at the elevated (10 °C) temperature. Previous brook trout studies have similarly found decreased rates of survival with elevated rearing temperatures ranging from 9.4 to 24 °C (Hokanson et al. 1973; Marten 1992; Baird et al. 2002); in some instances, maximum survival rates have ranged from 77 to 90% with an average maxima of 83% on optimal (current) rearing temperatures (Hokanson et al. 1973). Given that early life and development are universally thermally sensitive (Dahlke et al. 2020), it is possible that brook trout are experiencing too great of a thermal bottleneck at these temperatures for us to be able to detect differences in strain survival. It is also possible that the two populations responded similarly to the ambient and elevated temperature treatments due to their shared genetic ancestry.

Elevated rearing temperatures above the species' thermal optimum are known to reduce survival in brook trout (Hokanson et al. 1973; Baird et al. 2002). Despite this reduction, increased rearing temperatures are used to increase speed of development and manipulate the timing of fish production (Marten 1992; Fraser et al. 2019), to induce triploidy and sterilize fish for release (Galbreath and Samples 2000), and to prepare individuals for post release environments with elevated water temperatures (Cook et al. 2018a, b; Warriner et al. 2020; Durtsche et al. 2021; Penney

et al. 2022). It is thought that elevated temperatures induce eggs to hatch in an underdeveloped state due to accelerated activity and development of the hatch gland, compared to fish reared on temperatures that are cooler and closer to optimal (Hayes et al. 1953). This could be one cause of increased mortality, particularly at the emergent fry stage, as found in our study. Increased metabolic activity is thought to be another factor in the reduction of survival as embryos experience an increased oxygen demand and production of waste products (Cingi et al. 2010; Cook et al. 2018a). Increased metabolic wastes have also been linked to occurrences of blue sac disease (Madison et al. 2020), and could be another source of mortality for fish reared on elevated temperatures. Nonetheless, while warmer waters have been found to have lower levels of dissolved oxygen (Irby et al. 2018), this variable was controlled for in our study (maintained above 9 mg L^{-1} across both treatments (Cook et al. 2018a,b)); and water changes occurred every day to minimize waste accumulation. Instead, we posit that increased instances of blue sac disease for fish reared on elevated temperatures in our system was most reflective of temperature conditions as opposed to another environmental factor. While fertilization success is impacted by elevated temperatures (Cingi et al. 2010), it is important to note that differences in survival in our study were not impacted by temperature at fertilization, as both treatment groups were fertilized with cool and consistent water temperatures before being moved into incubation stacks. Additional studies have found the effects of increased temperatures on survival lessened after fish reach the eyed stage suggesting that manipulating development through rearing temperature after this point should not be harmful for embryos (Hokanson et al. 1973; Marten 1992); however, our results do not support this assertion as we found significant interaction between temperature and stage with reductions in survival occurring at the pre-exogenous feed and emergent fry stages at elevated temperatures.

Domestication and egg-to-fry survival

Contrary to our predictions, $F_{>25}$ Hill's Lake fish did not exhibit higher levels of survival under optimal hatchery-rearing conditions compared to F_1 Scott Lake families. Furthermore, F_1 Scott Lake fish did not fare better on elevated rearing temperature (which would have been indicative of retained plasticity from more recent generational exposure to unpredictable environments). Previous studies have shown that wild fish have greater thermal tolerance than hatchery fish possibly due to their increased genetic diversity and retaining plasticity due to their naturally varying environment (Carline and Machung 2001). Our results do not support these findings for our system as we found no interactions between strain and temperature on survival, suggesting that the F_1 Scott Lake strain was as equally as sensitive as the $F_{>25}$ Hill's Lake strain under ambient and elevated temperatures. This is consistent with the shared genetic ancestry between the two populations and suggests that neither multiple generations in captivity (Hill's Lake) nor more than ten generations of naturalized recruitment (Scott Lake) has significantly altered the thermal performance of these populations. In a related study, eggs and embryos from Scott Lake and Stringer Lake, another wild-origin brook trout populations from Algonquin Park with substantial Hill's Lake ancestry, exhibited similar respirometry and thermal performance, in contrast to other wild-origin Algonquin Park populations (Cook et al. 2018a,b).

Recent research has shown that one generation in captivity is able to elicit phenotypic change resulting in no phenotypic differences between F_1 and $F_{>1}$ generations of fish under captive conditions (Farquharson 2020; Gossieaux et al. 2020). Therefore, our results partially align with the findings of Fraser et al. (2019), suggesting captivity-driven changes can occur within a small number of generations and phenotypic plasticity may decrease after several generations in captivity (LaCava et al. 2023). Previous reviews have also identified phenotypic changes in one generation (Mason et al. 2013) but, as outlined in Milla et al. (2021), true domestication requires multiple generations for domestic characteristics (e.g. growth and nutritional needs, reproductive performance, immune function, and stress response) to emerge fully; essentially, rates of phenotypic change due to domestication are trait specific (Fraser 2008). Based on the lack of strain-temperature interactions and strain fixed effects, it seems likely that early offspring survival is one of the first phenotypic changes brought about by captivity, although not necessarily due to thermal stress.

Temperature and strain interact to increase the instance and types of malformation

We found no significant difference in the likelihood of malformations between strains of brook trout under either temperature regime, which was contrary to our predictions. Although past studies have found hatchery populations to have higher levels of malformations in more domesticated individuals (McDermid et al. 2010; Crichigno et al. 2021), this was not the case in our study. Instead, elevated rearing temperatures differentially impacted the F_1 Scott Lake strain, with the newly captive generation being more sensitive to elevate than current temperatures, also having a greater total number and likelihood of malformed individuals. Our results do not support our predictions that F_1 Scott Lake fish would be more robust and exhibit greater plasticity when faced with environmental stressors. It also does not support our predictions that $F_{>25}$ Hill's Lake fish would be of lower quality overall due to either the accumulation of genetic deleterious alleles or from a loss of plasticity from prolonged time in captivity as they did not exhibit a loss of quality or increased thermal sensitivity.

Elevated temperatures coupled with genetic factors are known to bring about various types of malformations (Branson and Turnbull 2008), and hatchery managers experience loss of biomass not necessarily in numbers but in quality when considering individuals with developmental malformations (Ihssen 1978). While it is not recommended that families producing high numbers of malformed individuals be integrated into broodstock (Sneddon et al. 2016), hatchery managers may not be afforded the luxury of removing breeding adults as it has been shown that low numbers of breeding adults in captive populations can reduce both genetic and phenotypic diversity which may have negative effects on the wild populations that are being supplemented (Perez-Enriquez et al. 1999; O'Sullivan et al. 2020).

Our study was one of the first to investigate the prevalence of various types of malformations in salmonids as current literature tends to focus on one specific type of malformation or malformations in one area of the body (i.e., vertebral malformations, head and jaw malformations, or gametal fusion). In our study, spinal (or vertebral) malformations were generally more common than other categories across temperature and hatchery generations; these malformations have been found to have a strong genetic basis (as well as being caused by nutritional deficiencies in the diet of developing fish) (Branson and Turnbull 2008). Spinal malformations have varying degrees of severity and often do not impact growth and survival but may impact condition factor and severely impact quality of produced fish (Fraser et al. 2015). With no reduction in growth and survival, it is possible families producing large numbers of fish with spinal malformations go unnoticed, contributing to the persistence of the responsible genotypes within domesticated breeding stocks as egg quality is often determined by egg survival (Bromage et al. 1992; Olk et al. 2019). Additionally, spinal malformations showed significant increases under elevated rearing temperatures, irrespective of strain, suggesting a strong environmental driver of this malformation type.

Instances of twinning and (rare) triplets, which we categorized as gametal fusion, were more common in our highly captive strain under both temperature regimes. Similar to spinal malformations, twinning is thought to also have a strong genetic basis and these greater proportions could have been a result of unintentional inbreeding within the hatchery system (Nowosad and Kucharczyk 2019). Nevertheless, elevated temperatures have been used to induce twinning in fish (e.g., Atlantic salmon; Fjelldal et al. 2016), and studies consistently show a higher instance of fusion malformations in heat-treated groups (Yamamoto et al. 1998; Owusu-Frimpong and Hargreaves 2000). Higher instances of gametal fusion in highly domestic fishes suggest the need to investigate existing broodstocks still displaying these developmental malformations and determine if genetic, environmental, or GxE interactions are the most likely source (Christie et al. 2016). Finally, blue sac disease is a common phenomenon that occurs when fish experience a metabolic change in response to environmental conditions like elevated temperatures (Madison et al. 2020). Although its expression was not influenced by temperatures in our study, it is a known occurrence in brook trout that has been found to have bacterial (*Aeromonas hydrophila*) and environmental causes such as crowding stress and nitrogenous wastes (Kayiş et al. 2015).

Our findings of F_1 Scott Lake fish exhibiting both a higher likelihood of malformation and greater

number of malformations when reared under elevated temperatures suggest either that Scott Lake fish are more thermally sensitive than the longer-captive strain in terms of offspring development, or that the Hill's Lake strain has lost some degree of phenotypic plasticity after multiple hatchery generations (LaCava et al. 2023). It is thought that increased handling stress on eggs, as well as elevated temperatures, can disrupt genetically determined developmental patterns and morphology (Brown et al. 2010). The F_1 generation may therefore have yet to adapt to increased disturbance of hatchery care and rearing densities (Kayiş et al. 2015). Interestingly, $F_{>25}$ Hill's Lake strain did not show an inability to cope with a novel environmental stressor, as would be indicated by lower thermal sensitivity in offspring development. A possible explanation is the Hill's Lake strain originating from brook trout in Pennsylvania (OMNRF 2005), and therefore could be considered a warm-adapted strain (Stitt et al. 2014). Additionally, Hill's Lake fish are typically used in studies as the representative "domesticated" strain in comparison to others (e.g., McDermid et al. 2012; Stitt et al. 2014; Howell et al. 2022). The Hill's Lake ancestry of the Scott Lake strain has likely conferred their thermal performance to a large, though incomplete degree, with time in captivity having an effect on certain traits as well. Taking these factors into consideration, future studies should consider investigating thermal sensitivity of an intermediately-captive ($F1 < F_n < F_{>25}$) strain from the same source population to determine if this thermal sensitivity decreases with time and adaptation occurs to other confounding hatchery stressors.

What is particularly interesting about our results is the differential expression of malformation types between strains. At 7 °C, F_1 Scott Lake fish showed significantly more larvae with blue sac disease and spinal malformations than rates of gametal fusion, while $F_{>25}$ Hill's Lake fish showed no differences at all between these same types. At 10 °C, F_1 Scott Lake fish showed no significant difference in the number of larvae with blue sac disease and spinal malformations, while $F_{>25}$ Hill's Lake fish had significantly more individuals with spinal malformations than blue sac disease. It is possible that these differences are driven by the increased thermal sensitivity that has been shown by F_1 Scott Lake fish. While our study only considered malformations that were easily identified upon visual inspection, future research should consider detailed imaging of fry to determine if malformations not easily identified visually are present (i.e. cardiovascular, head and jaw, and gill malformations (Branson and Turnbull 2008)). A more complete analysis of malformed individuals to identify possible genetic sources of congenital malformations within brood stocks would allow for the breeding of higher quality individuals.

Conclusion

With concerns for the effects stocked fishes have on the production in natural systems (Ersbak and Haase 1983; Einum and Fleming 2001; Kostow 2004; Gossieaux et al. 2020), characterizing the implications of warming water temperatures on the survival and quality of stocked fish in wild and hatchery settings, and with varying generations spent in captivity, is advisable for predicting the success of such programs (Einum and Fleming 2001; Venditti et al. 2018; O'Sullivan et al. 2020). As with other studies (Marten 1992; Yamamoto et al. 1996; Acornley 1999; Chezik et al. 2014) our own has shown that elevated temperatures affect offspring survival of cold-adapted freshwater salmonids. However, we additionally demonstrate certain developmental stages to be more sensitive to warmer temperatures, significantly reducing survival at the pre-exogenous feed and emergent fry stages. We also show temperature stressors to equally affect early survival of different strains of contrasting number of generations, but to have a greater impact on fry quality of newer captive strains. Breeding fish with varying generations in captivity has been suggested as a mitigation effect for domestication selection (i.e., mitigate genetic loss and slow down adaptation to captivity; Milla et al. 2021). Our results imply that this practice may not prevent the occurrence of environmentally induced malformations, despite the recognized genetic benefits (Frankham 2008; Fraser 2008; Fisch et al. 2015). Furthermore, the use of elevated temperatures to increase growth rates, prime offspring, and create multiple stocking events should be used with caution as it may come with production-efficiency and conservation costs that will need to be carefully titrated against the benefits of this practice.

Data availability

All data and supplementary materials are available from the corresponding author upon request.

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Ethics approval The authors declare all research to be completed in compliance with animal care standards and regulations.

Conflict of interest The authors declare no competing interests.

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